Docket No.: AREX-P03-004
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:	Madiyalakan *et al.*	Confirmation No.:	6693
Application No.:	09/376,604	Art Unit:	1643
Filed:	August 18, 1999		
For:	THERAPEUTIC COMPOSITIONS THAT ALTER THE IMMUNE RESPONSE	Examiner:	K. A. Canella

DECLARATION UNDER 37 C.F.R. § 1.132 OF BIRGIT SCHULTES

MS Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Birgit C. Schultes, Ph.D., of 12 Monadnock Road, Arlington, MA, hereby declare and state as follows:

1. I am Senior Director of Research at Unither Pharmaceuticals. I am also an inventor of the subject application. I have been conducting research in the field of tumor immunology for approximately 16 years. A copy of my *curriculum vitae* was enclosed with a previous Rule 132 Declaration submitted on November 28, 2005.

2. **Exhibit A** depicts the results of experiments carried out by me or under my direction. In these experiments, human peripheral blood leukocytes (PBLs) were purified from three HLA-matched healthy donors. From these purified PBLs, about 70-85% pure human monocytes and about 80-90% pure human T-cells were separately generated using negative selection with various antibodies. The purified monocytes were then used to generate immature dendritic cells (DCs) by culturing in cytokines (*e.g.*, GM-CSF and IL-4). Immature dendritic cells were then loaded with two different antigen-antibody complexes, namely the B43.13 / CA 125 complex and the AR9.6 / CA 125 complex. AR9.6, like B43.13, is a murine monoclonal IgG1 antibody against CA 125; however, AR9.6 binds a different CA 125 epitope than B43.13. Immature DCs loaded with either of the two antigen-antibody complexes (or as controls, loaded with either CA 125 alone, B43.13 alone, AR9.6 alone, or medium) were further matured with TNF-α and IFN-α, and were used to stimulate the purified T-cells. T-cell activation was subsequently assessed using two independent assays, the Intracellular Cytokine (ICC) staining for IFN-γ, or the Cytotoxic T Lymphocyte (CTL) Assay for killing CA 125-positive ovarian cancer cells NIH:OVCAR-3 (ATCC).

3. The first and the second graphs in **Exhibit A** show that, based on the CTL assay, both the B43.13 / CA 125 complex, and the AR9.6 / CA 125 complex significantly stimulated T-cell activation after 3 or 4 rounds of stimulation compared to either antibody alone, antigen alone, or the negative control (media alone).

4. The third, fourth, and fifth graphs in **Exhibit A** show that, based on the ICC assay, both the B43.13 / CA 125 complex, and the AR9.6 / CA 125 complex significantly stimulated T-cell activation in all three donors after 4 rounds of stimulation compared to either antibody alone, antigen alone, or the negative control (media alone).

5. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Dated: Feb. 8, 2007

Signature: _____
Birgit C. Schultes, Ph.D.